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ABSTRACT:

Atrial peptide analogs having formula (I), wherein R1is Ser or D-Ser; R4 is Phe or desR4; R5 is Gly or desR5; R6 is Gly or des R6; R8 is Met, Nle, Nva or Ile; R12 is Gly or D-Ala; R16 is Gly or desR16; R17 is Leu or desR17; R18 is Gly or desR18; R22 is Phe or desR22; R23 is Arg, Arg-Tyr or desR23; Q is S or CH2; Q' is S or CH2 and Y is OH or NHR, where R is H or lower alkyl. Either a D-isomer residue is present, or Q or Q' is CH2, or one or more of the residues in positions 4-6 and 16-18 is deleted. These analogs or pharmaceutically acceptable salts thereof, dispersed in a pharmaceutically acceptable liquid or solid carrier, can be administered to mammals for their natriuretic and diuretic activity or to relieve hypertension or to counteract congestive heart failure.

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(I)

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     CH2——— Q ————————————————————————————————————————————
     |  O
     |  ‖
R1-Ser-NH-CH-C-R4-R5-R6-Arg-R8-Asp-Arg-Ile-R12-Ala-Gln-Ser-
—— Q' ——————— CH2
              |  O
              |  ‖
R16-R17-R18-NH-CH-C-Asn-Ser-R22-R23-Y
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(57) Abstract

Atrial peptide analogs having formula (I), wherein R_1 is Ser or D-Ser; R_4 is Phe or desR_4; R_5 is Gly or desR_5; R_6 is Gly or des R_6; R_8 is Met, Nle, Nva or Ile; R_{12} is Gly or D-Ala; R_{16} is Gly or desR_{16}; R_{17} is Leu or desR_{17}; R_{18} is Gly or desR_{18}; R_{22} is Phe or desR_{22}; R_{23} is Arg, Arg-Tyr or desR_{23}; Q is S or CH_2; Q' is S or CH_2 and Y is OH or NHR, where R is H or lower alkyl. Either a D-isomer residue is present, or Q or Q' is CH_2, or one or more of the residues in positions 4-6 and 16-18 is deleted. These analogs or pharmaceutically acceptable salts thereof, dispersed in a pharmaceutically acceptable liquid or solid carrier, can be administered to mammals for their natriuretic and diuretic activity or to relieve hypertension or to counteract congestive heart failure.

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ATRIAL PEPTIDE ANALOGS

This invention is directed to peptides related to atrial peptides and to methods for pharmaceutical treatment of mammals using such peptides. More specifically, the invention relates to analogs of atriopeptin I and atriopeptin II, to pharmaceutical compositions containing such analogs and to methods of treatment of mammals using such analogs.

BACKGROUND OF THE INVENTION

Atriopeptins I and II were characterized by M. G. Currie et al. and are described in the *Science* issue of January 6, 1984. Atriopeptin I has the formula: Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-OH wherein there is a bridging bond between the sulfhydryl groups of the two cysteinyl amino acid residues. Atriopeptin II has the same 21 residues plus the residues Phe and Arg at the C-terminal at positions 22 and 23, respectively.

Atriopeptins I and II (APN-I and APN-II) exhibit potent effects on kidney function and regional vascular resistance; they have natriuretic, diuretic and smooth muscle relaxant activities.

SUMMARY OF THE INVENTION

Analogs of the 21- and 23- residue peptides atriopeptin I and II have been found which are more potent and/or longer acting than either atriopeptin I or II in functioning as a diuretic or as a smooth muscle relaxant. The peptide analogs have the formula:

$$\begin{array}{l} \quad\quad\quad\; CH_2 \text{———} Q \text{—————————————} \\ \quad\quad\quad\quad\; | \quad\; O \\ R_1\text{-Ser-NH-CH-}\overset{\|}{C}\text{-}R_4\text{-}R_5\text{-}R_6\text{-Arg-}R_8\text{-Asp-Arg-Ile-}R_{12}\text{-Ala-Gln-Ser-} \\ \text{——} Q \text{————} CH_2 \\ \quad\quad\quad\quad\quad\quad\quad\; | \quad\; O \\ R_{16}\text{-}R_{17}\text{-}R_{18}\text{-NH-CH-}\overset{\|}{C}\text{-Asn-Ser-}R_{22}\text{-}R_{23}\text{-Y} \end{array}$$

wherein R_1 is Ser or D-Ser; R_4 is Phe or desR_4; R_5 is Gly or desR_5; R_6 is Gly or desR_6, $R_8$is Ile, Nle, Nva or Met; R_{12} is Gly or D-Ala; R_{16} is Gly or desR_{16};R_{17} is Leu or desR_{17}; R_{18} is Gly or desR_{18}; R_{22} is Phe or desR_{22}; R_{23} is Arg, Arg-Tyr or desR_{23}; Q' is S or CH_2; Q is S or CH_2 and Y is

OH or NHR, where R is H or lower alkyl, provided that either a D-isomer residue is present, or Q or Q' is CH_2, or one or more of the residues in positions 4-6 and 16-18 is deleted.

Pharmaceutical compositions in accordance with the invention include such atrial peptide analogs, or nontoxic addition salts thereof, dispersed in a pharmaceutically acceptable liquid or solid carrier. The administration of such analogs or pharmaceutically acceptable addition salts thereof to mammals in accordance with the invention may be carried out for the regulation of urinary discharge, to relax intestinal smooth muscles, to relieve hypertension or to counteract congestive heart failure.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The nomenclature used to define the peptides is that specified by Schroder & Lubke, "The Peptides", Academic Press (1965) wherein, in accordance with conventional representation, the amino group appears to the left and the carboxyl group to the right. Where the amino acid residue has isomeric forms, it is the L-form of the amino acid that is represented unless otherwise expressly indicated.

The invention provides atrial peptide analogs having the following formula:

$$
\begin{array}{l}
\qquad\qquad\quad CH_2 \text{———} Q \text{————————————————————} \\
\qquad\qquad\quad | \quad\;\; O \\
\qquad\qquad\quad | \quad\;\; \| \\
R_1\text{-Ser-NH-CH-C-}R_4\text{-}R_5\text{-}R_6\text{-Arg-}R_8\text{-Asp-Arg-Ile-}R_{12}\text{-Ala-Gln-Ser-} \\
\text{—— Q' ————} \quad CH_2 \\
\qquad\qquad\qquad\quad\;\; | \quad\;\; O \\
\qquad\qquad\qquad\quad\;\; | \quad\;\; \| \\
R_{16}\text{-}R_{17}\text{-}R_{18}\text{-NH-CH-C-Asn-Ser-}R_{22}\text{-}R_{23}\text{-Y}
\end{array}
$$

wherein R_1 is Ser or D-Ser; R_4 is Phe or desR_4; R_5 is Gly or desR_5; R_6 is Gly or desR_6; R_8 is Met, Nle, Nva or Ile; R_{12} is Gly or D-Ala; R_{16} is Gly or desR_{16}; R_{17} is Leu or desR_{17}; R_{18} is Gly or desR_{18}; R_{22} is Phe or desR_{22}; R_{23} is Arg, Arg-Tyr or desR_{23}; Q is S or CH_2; Q' is S or CH_2 and Y is OH or NHR, where R is H or lower alkyl, provided that either a D-isomer residue is present, or Q or Q' is CH_2, or one or more of the residues in positions 4-6

and 16-18 is deleted. The preferred atrial peptide analogs include a disulfide bridge between the sulfhydryl groups of Cys residues; however, other analogs having an equivalent cyclizing bond are also biologically potent. Preferably, not more than one of the residues in positions 4-6 and not more than one of the residues in positions 16-18 is deleted.

The preferred peptides can be synthesized by a suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution addition. For example, the techniques of exclusively solid-state synthesis are set forth in the textbook "Solid-Phase Peptide Synthesis", Stewart & Young, Freeman & Co., San Francisco, 1969 and are exemplified by the disclosure of U.S. Patent No. 4,105,603, issued August 8, 1978 to Vale et al. The fragment condensation method of synthesis is exemplified in U.S. Patent No. 3,972,859 (August 3, 1976). Other available syntheses are exemplified by U.S. Patent No. 3,842,067 (October 15, 1974) and U.S. Patent No. 3,862,925 (January 28, 1975). Synthesis by the use of recombinant DNA techniques may also be used when no unnatural residues are present and should be understood to include the suitable employment of a structural gene coding for the desired form of analog. The synthetic peptide may be obtained by transforming a microorganism using an expression vector including a promoter and operator together with such structural gene and causing such transformed microorganism to express the peptide. A non-human animal may also be used to produce the peptide by gene-farming using such a structural gene and the general techniques set forth in U.S. Patent No. 4,276,282 issued June 30, 1981 or using microinjection of embryos as described in WO83/01783 published 26 May 1983 and WO82/04443 published 23 December 1982. The synthetic peptide is then suitably recovered from the animal by extraction from sera or the like.

Common to chemical syntheses of the preferred peptides is the protection of the labile side chain groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed. Usually also common is the protection of an alpha-amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the alpha-amino protecting group to allow subsequent reaction to take place at that location. Accordingly, it is common that, as a step in the synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with various of these residues linked to the side-chain protecting groups.

Also considered to be within the scope of the present invention are intermediates of the formula:

X^1-R_1(X^2)-Ser(X^2)-Cys(X^3)-R_4-R_5-R_6-Arg(X^4)-R_8-Asp(X^5)-Arg(X^4)-Ile-R_{12}-Ala-Gln(X^6)-Ser(X^2)-R_{16}-R_{17}-R_{18}-Cys(X^3)-Asn(X^6)-Ser(X^2)-R_{22}-R_{23}(X^4)-X^7

wherein: the R-groups are as hereinbefore defined; X^1 is either hydrogen or an a-amino protecting group. The a-amino protecting groups contemplated by X^1 are those known to be useful in the art in the step-wise synthesis of polypeptides. Among the classes of a-amino protecting groups covered by X^1 are (1) acyl-type protecting groups, such as formyl, trifluoroacetyl, phthalyl, p-toluenesulfonyl(Tos), benzensulfonyl, nitrophenylsulfenyl, tritylsulfenyl, o-nitrophenoxyacetyl, chloroacetyl, acetyl, and γ-chlorobutyryl; (2) aromatic urethan-type protecting groups, such as benzyloxycarbonyl(Z) and substituted Z, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl; (3) aliphatic urethan protecting groups, such as t-butyloxycarbonyl (BOC), diisopropylmethoxycarbonyl, isopropyloxycarbonyl,

ethoxycarbonyl, allyloxycarbonyl; (4) cycloalkyl urethan-type protecting groups, such as fluorenylmethyloxycarbonyl(FMOC), cyclopentyloxycarbonyl, adamantyloxycarbonyl,and cyclohexyloxycarbonyl; (5) thiourethan-type protecting groups, such as phenylthiocarbonyl; (6) alkyl-type protecting groups, such as triphenylmethyl(trityl), benzyl(Bzl); (7) trialkylsilane groups, such as trimethylsilane. The preferred a-amino protecting group is BOC.

X^2 is a protecting group for the hydroxyl group of Ser and is preferably selected from the class consisting of acetyl(Ac), benzoyl(Bz), tert-butyl, trityl, tetrahydropyranyl, benzyl ether(Bzl), 2,6-dichlorobenzyl and Z. The most preferred protecting group is Bzl. X^2 can be hydrogen, which means there is no protecting group on the hydroxyl group.

X^3 is a protecting group for Cys preferably selected from the class consisting of p-methoxybenzyl(MeOBzl), p-methylbenzyl, thioethyl, acetamidomethyl, trityl and Bzl. The most preferred protecting group is p-methoxybenzyl. X^3 can also be hydrogen, meaning that there is no protecting group on the sulfur.

X^4 is a protecting group for the guanidino group of Arg preferably selected from the class consisting of nitro, Tos, Z, adamantyloxycarbonyl and BOC, or is hydrogen. Tos is most preferred.

X^5 is hydrogen or an ester-forming protecting group for the ß-carboxyl group of Asp preferably selected from the class consisting of Bzl, 2,6-dichlorobenzyl(DCB), CBZ, methyl and ethyl. OBzl is most preferred.

X^6 is hydrogen or a protecting group for the amido group of Gln or Asn and is preferably xanthyl(Xan).

X^7 is selected from the class consisting of OH, OCH_3, amides, hydrazides and esters, including an

amide, a benzyl ester or a hydroxymethyl ester anchoring bond used in solid phase synthesis for linking to a solid resin support, represented by the formulae:

-NH-benzhydrylamine (BHA) resin support,

-NH-paramethylbenzhydrylamine (MBHA) resin support,

-O-CH_2-polystyrene resin support

and

O-CH_2-benzyl-polystyrene resin support

The polystyrene polymer is preferably a copolymer of styrene with about 0.5 to 2% divinyl benzene as a cross-linking agent, which causes the polystyrene polymer to be completely insoluble in certain organic solvents.

In the formula for the intermediate, at least one of X^1, X^2, X^3, X^4, X^5, X^6 and X^7 is a protecting group or resin support. In selecting a particular side chain protecting group to be used in the synthesis of the peptides, the following rules are followed: (a) the protecting group should be stable to the reagent and under the reaction conditions selected for removing the a-amino protecting group at each step of the synthesis, (b) the protecting group should retain its protecting properties and not be split off under coupling conditions and (c) the side chain protecting group must be removable, upon the completion of the synthesis containing the desired amino acid sequence, under reaction conditions that will not alter the peptide chain.

The peptides are preferably prepared using solid phase synthesis, such as that described by Merrifield, J. Am. Chem. Soc., 85, p 2149 (1964), although other equivalent chemical syntheses known in the art can also be used as previously mentioned. Solid-phase synthesis is commenced from the C-terminal end of the peptide by coupling a protected a-amino acid to a suitable resin. Such a starting material can be prepared by attaching a-amino- and guanidino-protected

Arg to a chloromethylated resin or to a hydroxymethyl resin. The preparation of the hydroxymethyl resin is described by Bodansky et al., Chem. Ind. (London) 38, 1597-98 (1966). Chloromethylated resins are commercially available from Bio Rad Laboratories, Richmond, California and from Lab. Systems, Inc. The preparation of such a resin is described by Stewart et al., "Solid Phase Peptide Synthesis" (Freeman & Co., San Francisco 1969), Chapter 1, pp 1-6.

Arg protected by BOC and by Tos is coupled to the chloromethylated polystyrene resin according to the procedure of Horiki et al. Chemistry Letters, pp 165-168, 1978. Following the coupling of BOC-(Tos)Arg to the resin support, the a-amino protecting group is removed, as by using trifluoroacetic acid(TFA) in methylene chloride, TFA alone or with HCl in dioxane. Preferably 50 weight % TFA in methylene chloride is used with 0-5 weight % 1,2 ethane dithiol. The deprotection is carried out at a temperature between about 0°C and room temperature. Other standard cleaving reagents and conditions for removal of specific a-amino protecting groups may be used as described in Schroder & Lubke, "The Peptides", 1 pp 72-75 (Academic Press 1965).

After removal of the a-amino protecting group of Arg, the remaining a-amino- and side chain-protected amino acids are coupled step-wise in the desired order to obtain the intermediate compound defined hereinbefore. As an alternative to adding each amino acid separately in the synthesis, some of them may be coupled to one another prior to addition to the solid phase reactor. The selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as coupling reagents are N,N'-dicyclohexyl carbodiimide (DCCI) and N,N'-diisopropylcarbodiimide(DICI).

The activating reagents used in the solid phase synthesis of the peptides are well known in the peptide

art. Examples of suitable activating reagents are: (1) carbodiimides, such as N,N'-diisopropyl carbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; (2) cyanamides such as N,N'-dibenzylcyanamide; (3) keteimines; (4) isoxazolium salts, such as N-ethyl-5-phenyl isoxazolium-3'-sulfonate; (5) monocyclic nitrogen-containing heterocyclic amides of aromatic character containing one through four nitrogens in the ring, such as imidazolides, pyrazolides, and 1,2,4-triazolides. Specific heterocyclic amides that are useful include N,N'-carbonyl diimidazole, N,N'-carbonyl-di-1,2,4-triazole; (6) alkoxylated acetylene, such as ethoxyacetylene; (7) reagents which form a mixed anhydride with the carboxyl moiety of the amino acid, such as ethylchloroformate and isobutylchloroformate and (8) nitrogen-containing heterocyclic compounds having a hydroxy group on one ring nitrogen, such as N-hydroxyphthalimide, N-hydroxysuccinimide and 1-hydroxybenzotriazole(HOBT). Other activating reagents and their use in peptide coupling are described by Schroder & Lubke, supra, in Chapter III and by Kapoor, J. Phar. Sci., 59, pp 1-27 (1970).

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in about a two-to fourfold excess, and the coupling is carried out in a medium of dimethylformamide(DMF):CH_2Cl_2 (1:1) or in DMF or CH_2Cl_2 alone. In instances where the coupling is carried out manually, the success of the coupling reaction at each stage of the synthesis is monitored by the ninhydrin reaction, as described by E. Kaiser et al., Anal. Biochem. 34, 595 (1970). In cases where incomplete coupling occurs, the coupling procedure is repeated before removal of the a-amino protecting group prior to the coupling of the next amino acid. The coupling reactions can be performed automatically, as on

a Beckman 990 automatic synthesizer, using a program such as that reported in Rivier et al., Biopolymers, 1978, 17, pp.1927-1938.

After the desired amino acid sequence has been completed, the intermediate peptide is removed from the resin support by treatment with a reagent, such as liquid hydrogen fluoride, which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups X^2, X^3, X^4, X^5 and X^6 and the a-amino protecting group X^1, to obtain the peptide in its linear form. The cyclic form of the peptide is obtained by oxidizing using a ferricyanide solution, preferably as described Rivier et al., Biopolymers, Vol. 17 (1978), 1927-38, or by air oxidation, or in accordance with other known procedures.

As an alternative route, the intermediate peptide may be separated from the resin support by alcoholysis after which the recovered C-terminal ester is converted to the acid by hydrolysis. Any side chain protecting groups may then be cleaved as previously described or by other known procedures, such as catalytic reduction (e.g. Pd on $BaSO_4$). When using hydrogen fluoride for cleaving, anisole and methylethyl sulfide are included in the reaction vessel for scavenging.

When the atrial peptide analogs are desired having the following general formula:

$$\overbrace{\mathrm{R_1\text{-}Ser\text{-}NH\text{-}\underset{}{CH}(CH_2\text{—}Q)\text{-}\overset{O}{\overset{\|}{C}}\text{-}R_4\text{-}R_5\text{-}R_6\text{-}Arg\text{-}R_8\text{-}Asp\text{-}Arg\text{-}Ile\text{-}R_{12}\text{-}Ala\text{-}Gln\text{-}Ser\text{-}}}$$

$$\mathrm{\text{—}Q'\text{—}CH_2}$$

$$\mathrm{R_{16}\text{-}R_{17}\text{-}R_{18}\text{-}NH\text{-}CH(CH_2)\text{-}\overset{O}{\overset{\|}{C}}\text{-}Asn\text{-}Ser\text{-}R_{22}\text{-}R_{23}\text{-}Y}$$

wherein Q and/or Q' is $-CH_2-$ are desired, they may be synthesized using the general principles set forth in any of the following U.S. patents: 4,115,554 (September 19, 1978); 4,133,805 (January 9, 1979); 4,140,767 (February 20, 1979); 4,161,521 (July 17, 1979); 4,191,754 (March 4, 1980);

4,238,481 (December 9, 1980); 4,244,947 (January 13, 1981); and 4,261,885 (April 14, 1981). Analogs having the disulfide linkage of cysteine residues replaced by $-CH_2-$ linkages are referred to as dicarba, e.g., [dicarba^{3,19}]-APN-I. If only one of the sulfhydryl groups is replaced by a CH_2-group, it is referred to as carba, e.g., [carba^{3}]-APN-I. Viewed from the aspect of the ultimate peptide, the location which would otherwise have been occupied by a Cys residue instead contains a residue of alpha-amino butyric acid(aBu). When preparing peptides having such a dicarba or carba-S linkage, the procedure set forth in U.S. Patent No. 4,161,521 is preferably employed.

The following Example sets forth the preferred method for synthesizing atrial peptide analogs by the solid-phase technique.

EXAMPLE I

The synthesis of the analog [D-Ala^{12}-Phe^{22}]-APN-I having the formula:
H-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-OH is conducted in a stepwise manner on a chloromethylated resin, such as LS-601 available from Lab Systems, Inc., containing 0.9 Meq Cl/gm. resin. Coupling of BOC-Phe to the washed resin is performed by the procedure set forth by Horiki et al., in Chemistry Letters (Chem. Soc. of Japan (1978) pp. 165-168, and it results in the substitution of about 0.35 mmol. Phe per gram of resin. All solvents that are used are carefully degassed, preferably by sparging with an inert gas, e.g., helium, to insure the absence of oxygen.

The coupling reaction is carried out in the reaction vessel of a Beckman Model 990 automatic peptide synthesizer which is programmed to perform the following general work cycle: (a) methylene chloride; (b) 60% trifluoroacetic acid in methylene chloride (2 times for

10 and 15 min resp.); (c) isopropyl alcohol wash; (d) 10% triethylamine in methylene chloride (2 times alternated with methanol wash); and (e) methylene chloride wash.

The washed resin (2g.) is stirred with 1.5 mmoles of BOC-Phe in methylene chloride and diisopropylcarbodiimide (1.5 mmoles) was added. The mixture was stirred at room temperature for 1 hour and the amino acid resin was then washed successively with methylene chloride, ethanol and methylene chloride (3 times each). The protected, attached amino acid was then cycled through steps (b) through (h) in the above wash program. The remaining amino acids (1.5 mmoles) are then coupled successively by the same cycle of events.

After deprotection and neutralization, the peptide chain is built step-by-step on the resin. Generally, one to two mmol. of BOC-protected amino acid in methylene chloride is used per gram of resin, plus one equivalent of 2 molar DCCI or diisopropylcarbodiimide in methylene chloride, for two hours. When BOC-Arg(Tos) or BOC-Asn(Xan) or BOC-Gln(Xan) is being coupled, a mixture of 90% DMF and methylene chloride is used. Bzl is used as the hydroxyl side-chain protecting group for Ser. P-nitrophenyl ester(ONp) can also be used to activate the carboxyl end of Asn, and BOC-Asn(ONp) is coupled overnight using one equivalent of HOBt in a 90% mixture of DMF and methylene chloride. Gln can also be similarly coupled. Alternatively, BOC-Asn and BOC-Gln are coupled using 1 meq. HOBt and 1 meq. DCCI in DMF. Tos is used to protect the guanidino group of Arg, and the aspartic carboxyl group is protected by OBzl. The amido group of Asn is protected by Xan. At the end of the synthesis, the following composition is obtained: BOC-Ser(Bzl)-Ser(Bzl)-Cys(MeOBzl)-Phe-Gly-Gly-Arg(Tos)-Ile-Asp(OBzl)-Arg(Tos)-Ile-D-Ala-Ala-Gln(Xan)-Ser(Bzl)-Gly-Leu-Gly-Cys(MeOBzl)-Asn(Xan)-Ser(Bzl)-Phe-O-CH_2-benzene-polystyrene resin support.

In order to cleave and deprotect the resulting protected peptide-resin, it is treated with 1.5 ml. anisole, and 15 ml. hydrogen fluoride (HF) per gram of peptide-resin, first at -20°C. for 20 min. and then at 0°C. for one-half hour. After elimination of the HF under high vacuum, the resin-peptide is washed with dry diethyl ether, and the peptide is then extracted with de-gassed 2N aqueous acetic acid and separated from the resin by filtration.

The cleaved and deprotected peptide is then air-oxidized under high dilution or is added dropwise to a potassium ferricyanide solution to form the disulfide bond between the Cys residues, as described by Rivier et al. in Biopolymers, Volume 17 (1978) pp. 1927-1938. After cyclization using the ferricyanide method, the peptide is chromatographed on both anion- and cation-exchange resins using the methods described in the Rivier et al. article and then lyophilized.

The peptide is then purified by gel permeation followed by semi-preparative HPLC as described in Rivier et al., Peptides: Structure and Biological Function (1979) pp. 125-128. The chromatographic fractions are carefully monitored by HPLC, and only the fractions showing substantial purity were pooled.

To check whether the precise composition was achieved, the analog is hydrolyzed in sealed evacuated tubes containing 4N methanesulfonic acid and 0.2% tryptamine for 24 hours at 110°C. Amino acid analyses of the hydrolysates using a Beckman 121 MB amino acid analyzer shows that the 22-residue peptide structure is obtained.

EXAMPLE II

The peptide [D-Ser^{1}, desAA^{4,5,6,16,17,18}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE III

The peptide [D-Ser^{1}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE IV

The peptide [D-Ser^{1},D-Ala^{12},Phe^{22}]-APN-I-NH_2, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-NH_2 is synthesized using the same general procedure as set forth in Example I but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE V

The peptide [D-Ser^{1}, desAA^{4,5,6,16,17,18}, D-Ala^{12}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE VI

The peptide [desAA^{4,5,6,16,17,18}, D-Ala^{12}, Phe^{22}]-APN-I, having the formula: H-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE VII

The peptide [desAA^{4,5,6,16,17,18}, Phe^{22}]-APN-I, having the formula: H-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE VIII

The peptide [D-Ser^{1}, desAA^{4,5,6,16,17,18}, D-Ala^{12}]-APN-II, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE IX

The peptide [D-Ser^{1}, D-Ala^{12}]-APN-II-NH_2 having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE X

The peptide [D-Ser^{1}, desAA^{4,5,6,16,17,18}, Met^{8}]-APN-II, having the formula: H-D-Ser-Ser-Cys-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XI

The peptide [D-Ser^{1}]-APN-II-NH_2, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XII

The peptide [desAA^{4,5,6,16,17,18}, D-Ala^{12}]-APN-II, having the formula: H-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XIII

The peptide [Nle^{8},D-Ala^{12}]-APN-II, having the formula: H-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Nle-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XIV

The peptide [Nva^{8},$desAA^{4,5,6,16,17,18}$]-APN-II-NH_2, having the formula: H-Ser-Ser-Cys-Arg-Nva-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-Phe-Arg-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XV

The peptide [D-Ser^{1}, $desAA^{4,5,6,16,17,18}$, D-Ala^{12}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XVI

The peptide [D-Ser^{1}, D-Ala^{12}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XVII

The peptide [D-Ser^{1}, $desAA^{4,5,6,16,17,18}$]-APN-I, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XVIII

The peptide [D-Ser^1]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XIX

The peptide [$desAA^{4,5,6,16,17,18}$, Met^8, D-Ala^{12}]-APN-I, having the formula: H-Ser-Ser-Cys-Arg-Met-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XX

The peptide [D-Ala^{12}]-APN-I, having the formula: H-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXI

The peptide [$desAA^{4,5,6,16,17,18}$]-APN-I-NH_2, having the formula: H-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXII

The peptide [$dicarba^{3,19}$]-APN-I, having the formula: H-Ser-Ser-aBu-Phe-Gly-Gly-Arg-Ile-Asp-Arg-

CH_2 ———— CH_2

Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-aBu-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I; however the cyclizing technique set forth in U.S. Patent No. 4,161,521 is employed to

provide the linkage between the moieties which occupy positions-3 and 19 in the ultimate peptide. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXIII

The peptide [desAA^{4,5,6,16,17,18}, dicarba^{3,19}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXIV

The peptide [D-Ala^{12}, dicarba^{3,19}]-APN-I-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing an MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXV

The peptide [desAA^{4,5,6,16,17,18}, Met^{8}, D-Ala^{12}, dicarba^{3,19}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXVI

The peptide [D-Ser^{1}, Met^{8},dicarba^{3,19}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXVII

The peptide [dicarba^{3,19}]-APN-II-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing an MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXVIII

The peptide [desAA$^{4, 5,6,16,17,18}$, Met^{8}, dicarba^{3,19}]-APN-II is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXIX

The peptide [desAA^{4,5,6,16,17,18}, Nle^{8}, D-Ala^{12}, dicarba^{3,19}]-APN-II is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXX

The peptide [D-Ala^{12}, dicarba^{3,19}]-APN-II-NH$_2$ is synthesized using the same general procedure as set forth in Example XXII but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXI

The peptide [D-Ser^{1}, dicarba^{3,19}]-APN-II is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXII

The peptide [dicarba^{3,19}, Phe^{22}]-APN-I-NH$_2$ is synthesized using the same general procedure as set forth in Example XXII but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXIII

The peptide [desAA^{4,5,6,16,17,18}, Met^{8}, dicarba^{3,19}, Phe^{22}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXIV

The peptide [dicarba^{3,19}, Met^{8}, D-Ala^{12}, Phe^{22}]-APN-I-NH$_2$ is synthesized using the same general procedure as set forth in Example XXII but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXV

The peptide [D-Ser^1, $desAA^{4,5,6,16,17,18}$, D-Ala^{12}]-APN-I-$NHCH_3$, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Cys-Asn-Ser-$NHCH_3$ is synthesized using the same general procedure as set forth in Example I but employing an N-methylbenzhydrylamine resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXVI

The peptide [D-Ser^1, D-Ala^{12}, Tyr^{24}]-APN-II, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXVII

The peptide [D-Ser^1, $desAA^{4,5,6,16,17,18}$]-APN-I-NH_2, having the formula: H-D-Ser-Ser-Cys-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Cys-Asn-Ser-NH_2 is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXVIII

The peptide [D-Ser^1, Met^8]-APN-I-$NHCH_3$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-$NHCH_3$ is synthesized using the same general procedure as set forth in Example XXXV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XXXIX

The peptide [$desAA^{4,5,6,16,17,18}$, $dicarba^{3,19}$, D-Ala^{12}, Phe^{22}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XL

The peptide [D-Ser^{1}, dicarba^{3,19}, Phe^{22}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLI

The peptide [D-Ser^{1}, desAA^{4,5,17,18}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLII

The peptide [D-Ser^{1}, carba^{3}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-aBu-Phe-Gly-Gly-Arg-

CH$_2$———————S

Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLIII

The peptide [D-Ser^{1}, desGly^{5}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLIV

The peptide [D-Ser^{1},D-Ala^{12},desGly^{5}, Phe^{22}]-APN-I-NH$_2$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-NH$_2$ is synthesized using the same general procedure as set forth in Example I but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLV

The peptide [D-Ser^{1}, desAA^{5,18},D-Ala^{12}, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLVI

The peptide [desAA^{5,16},D-Ala^{12}, Phe^{22}]-APN-I, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Leu-Gly-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLVII

The peptide [desAA^{5,18}, Phe^{22}]-APN-I, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-Phe-NH_2 is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE XLVIII

The peptide [D-Ser^{1}, desAA^{4,18}, D-Ala^{12}, Tyr^{24}]-APN-II, having the formula: H-D-Ser-Ser-Cys-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Phe-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE IL

The peptide [D-Ser^{1}, Tyr^{24}]-APN-II-NH_2 having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE L

The peptide [D-Ser^{1}, $desAA^{4,5,17,18}$, Phe^{22}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Cys-Asn-Ser-Phe-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LI

The peptide [D-Ser^{1},D-Ala^{12},Tyr^{24}]-APN-II-NH_2 is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LII

The peptide [$dicarba^{3,19}$, $desGly^{16}$,Phe^{22}]-APN-I-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LIII

The peptide [$desAA^{4,18}$, Met^{8}, $dicarba^{3,19}$, Phe^{22}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LIV

The peptide [$dicarba^{3,19}$, Met^{8}, D-Ala^{12}, $desGly^{16}$,Phe^{22}]-APN-I-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LV

The peptide [D-Ser^{1}, $desAA^{5,17}$,D-Ala^{12}]-APN-I-$NHCH_3$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Gly-Cys-Asn-Ser-$NHCH_3$ is synthesized using the same general procedure as set forth in Example I but employing an N-methylbenzhydrylamine resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LVI

The peptide [D-Ser^{1}, D-Ala^{12}, Tyr^{24}]-APN-II-NH$_2$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-NH$_2$ is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LVII

The peptide [D-Ser^{1},desAA^{5,16}]-APN-I-NH$_2$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Leu-Gly-Cys-Asn-Ser-NH$_2$ is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LVIII

The peptide [D-Ser^{1},Met^{8},desGly^{18}]-APN-I-NHCH$_3$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-NHCH$_3$ is synthesized using the same general procedure as set forth in Example XXXV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LIX

The peptide [desAA^{5,16}, dicarba^{3,19}, D-Ala^{12}, Phe^{22}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LX

The peptide [D-Ser^{1}, desAA^{5,17}, Met^{8}]-APN-II, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Met-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Gly-Cys-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXI

The peptide [D-Ser^{1},desGly^{5}]-APN-II-NH_2, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXII

The peptide [desAA^{5,18}, D-Ala^{12}]-APN-II, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXIII

The peptide [Nle^{8},D-Ala^{12},desGly^{18}]-APN-II-NH_2, having the formula: H-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Nle-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-Phe-Arg-NH_2 is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXIV

The peptide [Nva^{8},desAA^{5,16}]-APN-II-NH_2, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Nva-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Leu-Gly-Cys-Asn-Ser-Phe-Arg-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXV

The peptide [D-Ser^{1}, desAA^{5,17}, D-Ala^{12}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Gly-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXVI

The peptide [D-Ser^1, desGly^5, D-Ala^{12}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXVII

The peptide [D-Ser^1, desAA^{4,18}]-APN-I, having the formula: H-D-Ser-Ser-Cys-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXVIII

The peptide [D-Ser^1, desGly^{16}]-APN-I-NH$_2$, having the formula: H-D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Cys-Asn-Ser-NH$_2$ is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXIX

The peptide [desAA^{4,16},Met^8, D-Ala^{12}]-APN-I, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Met-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Leu-Gly-Cys-Asn-Ser-OH is synthesized using the same general procedure as set forth in Example I. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXX

The peptide [desGly^5,D-Ala^{12}]-APN-I-NH$_2$, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-D-Ala-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-NH$_2$ is synthesized using the same general procedure as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXI

The peptide [desAA^{5,18}]-APN-I-NH_2, having the formula: H-Ser-Ser-Cys-Phe-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Phe-Gly-Cys-Asn-Ser-NH_2 is synthesized as set forth in Example IV. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXII

The peptide [dicarba^{3,19}]-APN-II, having the formula: H-Ser-Ser-aBu-Phe-Gly-Gly-Arg-Ile-Asp-Arg-

CH_2 ——————— CH_2

Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-aBu-Asn-Ser-Phe-Arg-OH is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXIII

The peptide [desAA^{5,18}, dicarba^{3,19}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXIV

The peptide [desGly^{5},D-Ala^{12}, dicarba^{3,19}]-APN-I-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing an MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXV

The peptide [desAA^{4,18}, D-Ala^{12}, dicarba^{3,19}]-APN-I is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXVI

The peptide [D-Ser^{1}, Met^{8},dicarba^{3,19}]-APN-II is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXVII

The peptide [dicarba^{3,19},desGly^{18}]-APN-II-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing an MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXVIII

The peptide [desAA^{4,16}, Met^{8}, dicarba^{3,19}]-APN-II is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXIX

The peptide [desAA^{4,18}, Nle^{8}, D-Ala^{12}, dicarba^{3,19}]-APN-II is synthesized using the same general procedure as set forth in Example XXII. Amino acid analysis shows that the desired peptide structure is obtained.

EXAMPLE LXXX

The peptide [desGly^{5},D-Ala^{12}, dicarba^{3,19}]-APN-II-NH_2 is synthesized using the same general procedure as set forth in Example XXII but employing a MBHA resin. Amino acid analysis shows that the desired peptide structure is obtained.

In vivo testing of analogs of APN-I and APN-II shows that all of the peptides synthesized in the foregoing Examples exhibit natriuretic and diuretic activity. The testing is done using anesthetized Sprague-Dawley rats using the procedure as set forth in detail in an article by M.G. Currie, P.N.A.S. U.S.A. (1984). The analogs of APN-II are more potent than the comparable analogs of APN-I, and the iv administration of one microgram or less of the APN-II analogs induces an increase in urinary sodium excretion of 10 times or more.

In vitro testing is carried out with respect to these analogs to determine their activity as intestinal

smooth muscle relaxants and as vascular smooth muscle relaxants. The testing is performed using pre-contracted rabbit aorta strips and chick rectum strips, employing the procedure set forth in detail in M.G. Currie et al. Science, 221, 71 (1983). All of peptide analogs show potency as intestinal smooth muscle relaxants in the range of between about 10 nanograms and 100 nanograms. The APN-II analogs also show activity as vascular smooth muscle relaxants in about the same dosage range.

These peptides are believed to exhibit increased potency and increased duration of potency, as compared to APN-I and APN-II, in natriuretic and diuretic activity. They may be used to relieve hypertension or to counteract congestive heart failure by effecting a lowering of blood pressure. The administration of these analogs or the non-toxic addition salts thereof, combined with a pharmaceutically acceptable carrier to form a pharmaceutical composition, may be made to mammals, including humans, either intravenously, subcutaneously, intramuscularly, intranasally or orally, and a dosage of between about 1 microgram to about 10 milligrams per kilogram of body weight may be employed to take advantage of the natriuretic and diuretic activity. Moreover, the peptides may be employed for diagnostic purposes and/or in connection with surgery to serve as intestinal and/or vascular smooth muscle relaxants under the guidance of a physician who will be able to determine appropriate dosages from available test information and the case history of the patient in question.

Such peptides are often administered in the form of pharmaceutically acceptable nontoxic salts, such as acid addition salts or metal complexes, e.g., with zinc, iron, calcium, barium, magnesium, aluminum or the like (which are considered as addition salts for purposes of this application). Illustrative of such acid addition salts are hydrochloride, hydrobromide,

sulphate, phosphate, tannate, pamoate, oxalate, fumarate, gluconate, alginate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate, tartrate and the like. If the active ingredient is to be administered in tablet form, the tablet may contain a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate. If administration in liquid form is desired, sweetening and/or flavoring may be used, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected.

As previously indicated, the peptides should be administered under the guidance of a physician, and pharmaceutical compositions will usually contain the peptide in conjunction with a conventional, pharmaceutically-acceptable carrier. Usually, the dosage will be from about 2 to about 200 micrograms of the peptide per kilogram of the body weight of the host when the peptide is being used for other than its diuretic activity.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto. For example, substitutions and modifications at other positions in the peptide chain may be made that do not detract from the potency of the analogs, and such peptides are considered as being within the scope of the invention. Instead of the unsubstituted amide at the C-terminus, the amide may be substituted by lower alkyl or lower fluoroalkyl (1 to 4 carbon atoms).

WHAT IS CLAIMED IS:

1. A synthetic peptide or a nontoxic addition salt thereof having the formula:

$$R_1\text{-Ser-NH-}\overset{\overset{\displaystyle CH_2\text{———}Q\text{————————}}{|}}{CH}\text{-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-}R_4\text{-}R_5\text{-}R_6\text{-Arg-}R_8\text{-Asp-Arg-Ile-}R_{12}\text{-Ala-Gln-Ser-}$$

$$\text{——}Q'\text{————}\overset{\displaystyle CH_2}{|}$$

$$R_{16}\text{-}R_{17}\text{-}R_{18}\text{-NH-CH-}\overset{\overset{\displaystyle O}{\|}}{C}\text{-Asn-Ser-}R_{22}\text{-}R_{23}\text{-}Y$$

wherein R_1 is Ser or D-Ser; R_4 is Phe or desR_4; R_5 is Gly or desR_5; R_6 is Gly or desR_6; R_8 is Met, Nle, Nva or Ile, R_{12} is Gly or D-Ala; R_{16} is Gly or desR_{16}; R_{17} is Leu or desR_{17}; R_{18} is Gly or desR_{18}; R_{22} is Phe or desR_{22}; R_{23} is Arg or Arg-Tyr or desR_{23}; Q is S or CH_2; Q' is S or CH_2; and Y is OH or NHR where R is H or lower alkyl, provided that either R_1 is D-Ser or R_{12} is D-Ala or at least one of $R_4, R_5, R_6, R_{16}, R_{17}$ and R_{18} is deleted or Q or Q' is CH_2.

2. A peptide in accordance with Claim 1 wherein R_1 is D-Ser.

3. A peptide in accordance with Claim 1 wherein R_8 is Ile and R_{12} is D-Ala.

4. A peptide in accordance with Claim 2 wherein R_8 is Met and R_{12} is D-Ala.

5. A peptide in accordance with Claim 1 wherein R_4 is Phe and R_5 and R_6 are Gly.

6. A peptide in accordance with Claim 5 wherein R_{16} and R_{18} are Gly and R_{17} is Leu.

7. A peptide in accordance with Claim 1 wherein R_4 is Phe, R_5 is Gly, R_{16} is Gly, R_{17} is Leu and R_6 and R_{18} are deleted.

8. A peptide in accordance with Claim 1 wherein R_{22} is Phe.

9. A peptide in accordance with Claim 8 wherein R_{23} is Arg.

10. A peptide in accordance with Claim 1 wherein R_4 is Phe, R_5 is Gly, R_{17} is Leu, R_{18} is Gly and R_6 and R_{16} are deleted.

11. A peptide in accordance with Claim 1 wherein only one of R_4, R_5 and R_6 is deleted and only one of R_{16}, R_{17} and R_{18} is deleted.

12. A peptide in accordance with Claim 6 wherein R_8 is Met.

13. A peptide in accordance with Claim 6 wherein R_8 is Ile.

14. A peptide in accordance with Claim 7 wherein R_8 is Met.

15. A peptide in accordance with Claim 7 wherein R_8 is Ile.

16. A peptide in accordance with Claim 1 having the formula:

CH_2 ——— CH_2 ———————————————
| O
R_1-Ser-NH-CH-C-R_4-R_5-R_6-Arg-Ile-Asp-Arg-Ile-R_{12}-Ala-Gln-Ser-
—— CH_2 ——————— CH_2
| O
R_{16}-R_{17}-R_{18}-NH-CH-C-Asn-Ser-R_{22}-R_{23}-OH.

17. A peptide in accordance with Claim 1 having the formula:

D-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-OH

18. A pharmaceutical composition for increasing urinary discharge comprising an effective amount of a synthetic peptide, or a nontoxic addition salt thereof, in accordance with Claim 1, and a pharmaceutically acceptable liquid or solid carrier therefor.

19. A process for the manufacture of compounds defined by the formula(I):

CH_2 ——— Q ———————————————
R_1-Ser-NHCH-CO-R_4-R_5-R_6-Arg-R_8-Asp-Arg-Ile-R_{12}-Ala-Gln-Ser-
—— Q' ——————— CH_2
| O
R_{16}-R_{17}-R_{18}-NHCH-CO-Asn-Ser-R_{22}-R_{23}-Y

wherein R_1 is Ser or D-Ser; R_4 is Phe or desR_4; R_5 is Gly or desR_5; R_6 is Gly or desR_6; R_8 is Met, Nle, Nva or Ile; R_{12} is Gly or D-Ala; R_{16} is Gly or desR_{16}; R_{17} is Leu or desR_{17}; R_{18} is Gly or desR_{18}; R_{22} is Phe or desR_{22}; R_{23} is Arg or Arg-Tyr or desR_{23} and Y is OH or NHR where R is H or lower alkyl; provided that either R_1 is D-Ser or R_{12} is D-Ala or at least one of R_4, R_5, R_6, R_{16}, R_{17} and R_{18} is deleted; comprising (a) forming a peptide having at least one protective group and having the formula(II):

$$X^1\text{-}R_1(X^2)\text{-Ser}(X^2)\text{-NHCH}(\overset{(X_3^1)}{CH_2})\text{CO-}R_4\text{-}R_5\text{-}R_6\text{-Arg}(X^4)\text{-}R_8\text{-}$$
$$\text{Asp}(X^5)\text{-Arg}(X^4)\text{-Ile-}R_{12}\text{-Ala-Gln}(X^6)\text{-Ser}(X^2)\text{-}R_{16}\text{-}R_{17}\text{-}R_{18}\text{-}$$
$$\text{NHCH}(\overset{(X_3^1)}{CH_2})\text{CO-Asn}(X^6)\text{-Ser}(X^2)\text{-}R_{22}\text{-}R_{23}(X^4)\text{-}X^7$$

wherein X_1, X_2, X_4, X_5 and X_6 are each either hydrogen or a protective group, X_3 is either $-CH_2-$ or $S(X_3)$, with X_3 being a protecting group for sulfur, and X_7 is either a protective group or an anchoring bond to resin support or OH or NH_2; (b) splitting off the protective group or groups or anchoring bond from said peptide of the formula(II); (c) oxidizing said peptide to create a disulfide linkage when X_3 contains S and (d) if desired, converting a resulting peptide into a nontoxic addition salt thereof.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/00746

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

US CL. 260/112.5R; 514/11;514/13

INT. CL. -3- C07C 103/52; A61K 37/00

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	260/112.5R; 514/11;514/13

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A,P,	US, A, 4,508,712 Published 2 Apr. 1985 Needleman	1-18
A,P,	US, A, 4,496,544 Published 29 Jan. 1985 Needleman	1-18
A	N, Science, Vol. 221, Issued 1983, pages 71-73, Currie, etal.	1-18
A	N, Chemical Abstract, Vol. 94, Issued 1981, page 62919, De Bold, et al.	1-18
A	N, Chemical Abstract, Vol. 97, Issued 1982, page 175913 Trippodo, et al.	1-18
A	N, FEBS, 1268, Vol. 167, No. 2, Issued 1984, pages 352-357, Thibault, et al.	1-18

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
27 June 1985	10 JUL 1985
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	Delbert R. Phillips

FURTHER INFORMATION CONTINUED FROM THE FIRST SHEET
(Not for publication)

A	N,	Biochemical and Biophysical Research Communication, Vol. 118, Issued 1984, pages 131-139 Kangawa, et al.	1-18
A	N,	Biochemical and Biophysical Research Communications, Vol. 117, Issued 1983, pages 859-865 Flynn, et al	1-18
A	N,	Biochemical and Biophysical Research Communications, Vol. 119, Issued 1984, pages 524-529 Misono, etal.	1-18
A	N,	Science, Vol. 223, Issued 1984 pages 67-69, Currie, et al.	1-18

International Application No. PCT/US85/00746

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ...., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

I. Claims 1-18
II. Claim 19

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1 — 18
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.